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Research

Progressive sensitivity of trophic levels to warming underlies an elevational gradient in ant–aphid mutualism strength

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Although species interactions are often proposed to be stronger at lower latitudes and elevations, few studies have evaluated the mechanisms driving such patterns. In this study, we assessed whether, and by which mechanisms, abiotic changes associated with elevation altered the outcome of an ant–aphid protection mutualism. To do so, we characterized the multi-trophic interactions among the ant *Formica podzolica*, the aphid *Aphis varians*, and aphid natural enemies occurring on the plant *Chamerion angustifolium* within replicate high and low elevation valleys. Low (versus high) elevation sites had longer summers (snowmelt 13 days earlier) and were on average 1.1°C warmer and 41% drier throughout the year. At low elevations, individual ant colonies consumed approximately double the volume of carbohydrate baits, likely due to a higher foraging tempo, and possibly due to a greater demand for sugar- versus protein-rich resources (as indicated by stable isotope analysis). Wild aphid colonies at low elevations were visited by 1.4-fold more natural enemies (controlling for variation in aphid abundance), while experimental aphid colonies on potted plants were tended 52% more frequently by ants. As a result, ants increased aphid colony survival by 66% at low elevations but had no detectable effect at high elevations; at low (versus high) elevations aphid colonies without ants had lower survival, demonstrating stronger predator effects, while aphid colonies with ants had higher survival, demonstrating even stronger ant benefits. Analyses for the effects of mean summer temperature yielded qualitatively identical results to those based on elevation. Collectively, these findings support predictions for a greater sensitivity of higher trophic levels to warming and demonstrate how species interactions can vary across environmental gradients due to simultaneous changes in species traits and abundances across multiple trophic levels.

Keywords: ant–aphid mutualism, elevation, multi-trophic interactions

Introduction

Species interactions, from antagonisms to mutualisms, have been proposed to be stronger at lower elevations and towards the tropics, where temperatures are warmer and the climate is less seasonal (Schemske et al. 2009, Moreira et al. 2018). However, recent studies have questioned the generality of this pattern (Moles et al. 2011, Moles and Ollerton 2016), and we lack a clear mechanistic framework for predicting variation in species interactions along environmental gradients (Moreira et al. 2018). Because interspecific interactions are key determinants of species abundances and distributions, changes in these interactions can have widespread ecological and evolutionary effects (Aslan et al. 2013). Thus, understanding the factors that cause variation in species interactions is increasingly important for predicting how communities will be affected by climate change (Tylianakis et al. 2008, Gilman et al. 2010).

Tests for gradients in species interactions typically measure the strength of a focal interaction over space (Schemske et al. 2009, Moreira et al. 2018), but this approach does not yield an understanding of the mechanisms underlying such patterns (Moreira et al. 2018). Pairwise interactions should vary if the abiotic environment directly affects the traits or abundances of one or both interacting species. If species respond in parallel, pairwise interactions may remain unchanged. However, evidence suggests that species often respond heterogeneously to changes in the abiotic environment (Visser and Both 2005, Tylianakis et al. 2008, Both et al. 2009). In particular, changes in abiotic conditions may have stronger effects on higher than lower trophic levels, possibly due to organisms at higher trophic levels having relatively greater metabolic requirements or smaller population sizes (Petchey et al. 1999, Voigt et al. 2003, Vasseur and McCann 2005). As a result, trophic gradients in sensitivity to warming may be commonplace (Kishi et al. 2005, Barton et al. 2009), although the effect of warming on predator physiology (positive versus negative) is often variable (Tylianakis et al. 2008). In addition, gradients in pairwise interactions may be driven not only by environmental effects on the focal species, but also by variation in the multi-trophic food webs within which the interactions are embedded (Walther 2010, Mooney et al. 2016). Accordingly, a mechanistic understanding of gradients in species interactions requires documenting variation not only in interaction outcomes, but also in the abundances and traits of the interacting species, and the biotic contexts within which their interactions occurs (Gilman et al. 2010, Mooney et al. 2016).

Ant protection mutualisms are experimentally tractable multi-trophic interactions and, as such, are ideal systems for investigating the mechanisms driving clinal variation in species interactions. In these mutualisms, ants consume resources produced by myrmecophilous plants or insects (i.e. food bodies and extrafloral nectar or honeydew, respectively) and in exchange provide protection against natural enemies (i.e. herbivores or predators and parasitoids, respectively) (Way 1963, Janzen 1966). By doing so, ants alter the abundance, distribution, and evolution of their mutualist partners (Olmstead and Wood 1990, Abdala-Roberts et al. 2012, Pellissier et al. 2012) and can also influence associated plant and arthropod communities (Styrsky and Eubanks 2007, Schuldt et al. 2017). There is some evidence that ant protection mutualisms are stronger at lower elevations and latitudes (Koptur 1985, Olmstead and Wood 1990, Chamberlain and Holland 2009, Plowman et al. 2017). This pattern may be driven by the effects of the abiotic environment on the traits, abundances, and community composition not only of ants and their myrmecophilous partners, but also of their resources and natural enemies (Petry et al. 2012, Chamberlain et al. 2014, Staab et al. 2015, Mooney et al. 2016). Because ant protection mutualisms constitute multi-trophic interactions (involving mutualist plants or herbivores, natural enemies and ants), they thus provide the opportunity to test the specific prediction for stronger elevational effects on higher trophic levels.

In this study, we evaluated whether elevation altered the performance of an ant-tended aphid by mediating the activity of mutualist ants and the abundance of natural enemies. Based on the hypotheses for stronger species interactions at lower elevations and for a trophic gradient in sensitivity to warming (Voigt et al. 2003, Schemske et al. 2009, Moreira et al. 2018), we made three predictions: first, the top–down control by natural enemies (third trophic level) would increase at low elevations; second, the protection provided by mutualist ants (fourth trophic level) would also increase at low elevations; third, if ants (fourth trophic level) respond more to elevation than predators (third trophic level), the mutualistic services provided by ants to aphids – protection from predators – would increase at low elevations. To test these predictions, we assessed the interactions between the ant *Formica podzolica* (Hymenoptera: Formicidae) and the aphid *Aphis varians* (Hemiptera: Aphididae) feeding on fireweed *Chamerion angustifolium* within replicate high and low elevation valleys. In doing so, we provide a novel test for the multi-trophic basis of elevational gradients in species interactions.

Material and methods

Study sites

We conducted multiple complementary studies across four years (from June to August in 2009, 2010, 2012 and 2015) near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO (38°96'N, −106°99'W). In each year we sampled from the same ten sites (or a subset of these sites) in each of three 'low elevation' valleys (Cement Creek, Spring Creek, and Taylor River), with sites ranging in elevation from 2544 to 2748 m (2660 m ± 69 SD), and three 'high elevation' valleys (East River, Slate River, and Washington Gulch), with sites ranging in elevation from

2873 to 3327 m (2987 m ± 108 SD) (n = 30 low and 30 high elevation sites, for a total of 60; Fig. 1). Each valley was at least 3 km apart, with 17 km (Euclidean distances) separating the low and high elevation valleys. Along a linear transect in each valley, neighboring sites were separated by a minimum of 30 m, with the most distant sites separated by up to 2 500 m.

Although we did not collect climate data within our sites, we used the PRISM Climate Group Model (<www.prism.oregonstate.edu>) to estimate climatic variables for each site. Based on the data available, we estimated that the low elevation sites in 2009–2015 were 1.1°C warmer (3.5 ± 0.3°C versus 2.4 ± 0.2°C, respectively [mean annual temperature ± SD]) and 41% drier (576 ± 72 mm year^{-1} versus 981 ± 117 mm year^{-1}, respectively [mean annual precipitation ± SD]) as the result of a regional north–south aridity gradient (Petry et al. 2016). For the summer months (May–September) in particular, the low elevation sites were estimated to have been 1.6°C warmer (11.8 ± 0.4°C versus 10.2 ± 0.4°C, respectively [mean monthly temperature ± SD]), 29% drier (45 ± 3 mm month^{-1} versus 63 ± 6 mm month^{-1}, respectively [mean monthly precipitation ± SD]), and had a longer growing season (snowmelt 13 days earlier at low [131 ± 3 day of year] versus high [144 ± 4 day of year] elevation sites as estimated from a regional regression; Petry et al. 2016).

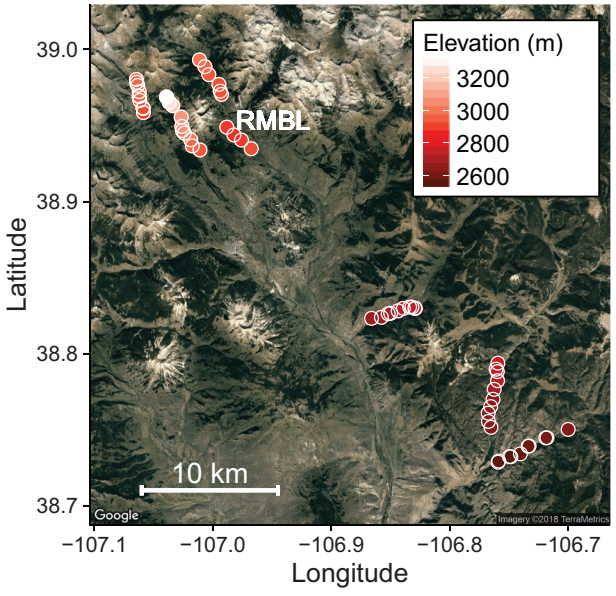

Figure 1. The distribution of sites in the three high (top left) and three low elevation valleys (bottom right), with elevation indicated by color. Low elevation sites were 1.1°C warmer (3.5 ± 0.3°C versus 2.4 ± 0.2°C, respectively [mean annual temperature ± SD]), 41% drier (576 ± 72 mm year^{-1} versus 981 ± 117 mm year^{-1}, respectively [mean annual precipitation ± SD]), and had a longer growing season (snowmelt 13 days earlier at low [131 ± 3 day of year] versus high [144 ± 4 day of year]).

Natural history

The ant *Formica podzolica* (Hymenoptera: Formicidae) forms colonies typically consisting of 5 000–40 000 workers and one or multiple queens, with colonies sometimes occupying multiple separate nests (Deslippe and Savolainen 1995, DeHeer and Herbers 2004). *Formica podzolica* commonly forages in plant canopies, where it preys on many arthropods and also consumes honeydew produced by aphids (Mooney and Tillberg 2005). One such aphid, *Aphis varians* (Hemiptera: Aphididae), feeds on fireweed (Onagraceae: *Chamerion angustifolium*), which is an herbaceous perennial plant that hosts four aphid species (*Aphis helianthi*, *A. salicariae*, *A. varians* and *Macrosiphum valerianae*) in the Rocky Mountains in central Colorado (Addicott 1978). Of these aphids, *A. varians* is the most abundant in this region and reaches peak abundances in mid-July (Addicott 1978). *Aphis varians* is also the aphid that is most frequently tended by ants (including *F. podzolica* and less frequently *Camponotus* spp., ants in the *F. rufa* species group, and *Tapinoma sessile*) (Addicott 1978).

Analytical approaches

Our tests for elevation effects were focused on a categorical (high versus low) classification for several reasons. Because climatic differences along this elevational gradient (Fig. 1) are enhanced by the regional north–south aridity gradient (Petry et al. 2016), the effects of elevation are much greater among valleys (high versus low) than within valleys, making elevation a poor proxy for climatic differences. While the PRISM Climate Group Model provides estimates of climatic conditions within our study area, the relatively low resolution of the model means that the 10 replicate sites within each valley can only be ascribed between two and four separate estimates of climatic conditions, resulting in a pseudo-replicated analysis. Nevertheless, we provide supplemental statistical analyses (presented in Supplementary material Appendix 2) using mean summer temperature (estimated from the PRISM Climate Group Model for May–September in 2009–2015) as a continuous predictor variable. Because analyses of temperature are pseudo-replicated, we emphasize our categorical analyses of elevation.

Variables assessed

Ant abundance and colony activity

To test for elevational differences in ant abundance and activity, we collected *F. podzolica* ants in pitfall traps in 2012 and 2015. Pitfall traps consisted of 50 ml plastic centrifuge tubes with 2.75 cm diameters that were filled with soapy water and placed flush with the ground surface. In 2015 each site contained two traps and was sampled twice (between 24 and 27 June and between 21 and 30 July) (full summary of response variables and sampling methods in Supplementary material Appendix 1 Table A1.1). In 2012 a subset of the sites (three sites distributed throughout each valley; n = 9 low

and 9 high elevation sites, for a total of 18) was sampled once between 2 July and 14 August, with 8–16 traps on a grid (depending on site size) (Supplementary material Appendix 1 Table A1.1). Within a site traps were separated by 3 m and placed at least 1 m away from nearby ant mounds. After deploying traps for 24–120 h (depending on ant accumulation rates), we counted the total number of *F. podzolica* in all traps within each site. To assess whether ant abundance in pitfall traps differed with elevation, we used separate linear mixed models (LMMs) for 2012 and 2015, which both included the number of *F. podzolica* collected per pitfall trap per day within a site (rates used to account for differences in sampling time; cube-root transformed to improve the normality of residuals) as the response variable. Elevation was included as a fixed effect, and valley nested within elevation was included as a random effect (full summary of statistical analyses in Supplementary material Appendix 1 Table A1.2). Any elevational differences in ant abundance could have been due to differences in ant colony density, colony size or forager activity.

To test for intraspecific differences in ant colony activity, we examined one focal *F. podzolica* ant nest within each site in 2010 (n = 30 low and 30 high elevation nests, for a total of 60) (Supplementary material Appendix 1 Table A1.1). Nest mounds were selected to be of approximately the same size, based on the surface area of the ground covered. For each nest mound we measured the longest and shortest diameters, which we used to calculate the area of the mound as the area of an ellipse. Nest mound surface area has previously been found to be positively correlated with worker number in *Formica* colonies (Liautard et al. 2003). To confirm that the nest mounds we selected were of approximately the same size, we tested for elevational differences in ant nest mound area (ln-transformed to improve normality), with elevation as a fixed effect and valley nested within elevation as a random effect (Supplementary material Appendix 1 Table A1.2). Because two high-elevation ant colonies were unusually large (area $>1\,m^2$ versus $0.28 \pm 0.13\,m^2$), we excluded them from this and all subsequent analyses, which did not qualitatively affect the result of this analysis. We evaluated ant activity by counting the number of ants on the mound surface on 7–9 separate occasions from 28 July to 24 August, likely providing a combined measure of the activity of foragers and of ants engaged in other activities (e.g. defense or nest construction) (Supplementary material Appendix 1 Table A1.1). We used a LMM to test for elevational differences in ant activity on the mound surface, with the mean number of ants observed on the mound across all dates (ln-transformed) as the response variable, elevation as a fixed effect, and valley nested within elevation as a random effect (Supplementary material Appendix 1 Table A1.2).

Ant diet as indicated by stable isotope analysis

We assessed whether ant diet varied with elevation by analyzing ant nitrogen and carbon stable isotopes. Elevational differences in resource availability or ant colony nutritional requirements could cause differences in ant diet measured using stable isotopes, which in turn could explain differences in ant interactions with aphids. To measure ant diet using stable isotopes, we collected 1–3 adults and pupae from a subset of the same focal ant colonies (4–5 mounds distributed throughout each valley; n = 28 total mounds) in August 2010 (Supplementary material Appendix 1 Table A1.1). Adult foraging ants were collected as they departed the mound surface (i.e. not returning foragers), and past work with this species has confirmed that only returning (not departing) foragers had full gasters (Mooney and Tillberg 2005). Thus, we used complete ants (without discharging their gasters) for all stable isotope analyses. The ants were dried at 60°C for 72 h before being ground to a fine powder with a bug grinding mill. Approximately 1 mg of this powder was packed into 5×9 mm tins for elemental analysis and mass spectrometry at the UC Irvine Stable Isotope Ratio and Mass Spectrometry Facility.

We measured the heavy: light isotopic ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$). Because nitrogen isotopic values become enriched at higher trophic levels, $\delta^{15}N$ can be used to determine ant trophic position and whether ant diets are primarily based on plant-based carbohydrates or arthropod prey (Mooney and Tillberg 2005, Tillberg et al. 2006). Although carbon isotopes show little fractionation with trophic level, they can differ among primary producers (e.g. between C3 versus C4 plants) and thus indicate whether ants consumed resources based on different food webs (Blüthgen et al. 2003, Tillberg et al. 2006). We also measured ant C and N dry weight concentrations ('percent C' and 'percent N'), which were used to calculate C:N ratios to assess the relative contribution of carbohydrate- and protein-based resources to ant diets. We predicted that if higher temperatures at low elevations increased ant activity, foragers might consume additional carbohydrates relative to proteins (lower $\delta^{15}N$ and higher C:N) as 'fuel' (Davidson 1997). Similarly, if the composition of sugar-rich resources in ant diets (e.g. aphid honeydew, floral nectar, or extrafloral nectar) varies with elevation, we predicted that there would be differences in ant trophic position or $\delta^{13}C$ (Blüthgen et al. 2003).

To test for elevational differences in ant stable isotopes, we constructed separate LMMs with the $\delta^{15}N$, $\delta^{13}C$, C:N ratio, percent C, and percent N as response variables. All models included elevation as a fixed effect and valley nested within elevation as a random effect. The models also included ant life stage (i.e. adult or pupa) as a fixed effect and ant colony nested within valley as a random effect (since adults and pupae were collected from the same nest mounds) (Supplementary material Appendix 1 Table A1.2).

Ant carbohydrate consumption as indicated by recruitment to baits

To further assess ant colony activity and diet, we examined ant consumption of carbohydrates from sets of artificial baits placed on the edge of each of the same focal nest mounds (n = 30 low and 30 high elevation mounds, for a total of 60) on three separate occasions (28 July, 11 and 17 August 2010) (Supplementary material Appendix 1 Table A1.1). Each set

consisted of three baits containing 8 ml of 10%, 20% and 30% honey solutions in 15 ml plastic centrifuge tubes, which were plugged with cotton wicks, as well as an identical water-filled tube (0% honey). These baits were deployed for approximately 24 h (range 20–27 h). When baits were collected, we recorded the number of ants feeding in each tube ('forager abundance'). To determine bait consumption rates (mg h^{-1}), we weighed baits before and after they were deployed to calculate the mass lost per hour. We corrected these consumption rates for evaporative water loss, measured from the water control (ants were not observed collecting water); if evaporation rates exceeded consumption rates (producing a negative adjusted consumption rate), we presumed that the actual consumption rate was zero. While the potential sample size in this design for each elevation and bait concentration was 90 (10 sites × 3 valleys per elevation × 3 sampling dates = 90), in some cases consumption rates or ant counts were missing, resulting in the following realized sample sizes: 76, 85 and 85 baits consisting of 10%, 20% and 30% honey (respectively) at low elevations, and 84, 83 and 84 baits consisting of 10%, 20% and 30% honey (respectively) at high elevations. Ant forager abundance in the baits was small relative to the number of ants in the colony, as indicated by the fact that the number of ants observed in baits was 8.5% and 4.2% of the number of ants observed on the mound surface at low and high elevations, respectively. In addition, because baits were placed immediately adjacent to the nest, and nests were selected to be similar in size, we assume that any differences in forager abundance in the baits were due solely to differences in ant foraging decisions, and did not reflect variation in forager availability.

We evaluated whether ant colony bait consumption rate, forager abundance in baits, and bait consumption per observed ant depended on elevation and sugar concentration. LMMs were used for both bait consumption rate and bait consumption rate per ant. We used a generalized linear mixed model (GLMM; for count data with non-normally distributed residuals) to assess forager abundance in the baits (observed once each time the baits were collected). The GLMM was fit with three possible distributions (Poisson, negative binomial, and negative binomial with a quasi-Poisson scale parameter), and we report results from the best-fitting model (negative binomial with a quasi-Poisson scale parameter), which was selected by comparing AIC values. To calculate bait consumption rate per ant, we divided consumption rates by forager abundances in the baits (with 1 added to all ant counts to include cases when ants were not observed). We ln + 1 transformed both the bait consumption rate and bait consumption rate per ant to improve the normality of residuals. All models included the main and interactive effects of elevation and sugar concentration, as well as the random effect of valley nested within elevation. Since we collected data at each mound on three separate dates throughout the season, we conducted a repeated measures analysis by including date as a fixed effect and mound nested within valley as a random effect (Supplementary material Appendix 1 Table A1.2). In all models a significant main effect of elevation would indicate an overall difference in ant feeding from baits, controlling for differences in sugar concentration. A significant elevation × sugar concentration interaction would indicate that elevation altered the strength of ant responses to changes in resource quality.

Effect of ants on aphids

To assess whether elevation altered aphid performance and the effects of ants on aphid performance, we evaluated the ant tending, survival, and growth of aphid colonies on potted fireweed plants placed adjacent to the same focal *F. podzolica* ant nest mounds in each site (Supplementary material Appendix 1 Table A1.1). Plants were grown from seed collected from within 1 km of the RMBL, thus controlling for any elevational effects on plant quality. The seeds were germinated in early May 2010, and seedlings were grown individually in 125 ml pots in a greenhouse at the University of California at Irvine. In mid-June the plants were transported to the RMBL and transplanted into 2 l pots with locally collected soil. Plants were watered every other day and fertilized once per week. On 8 August 2010, we added ten unwinged aphids (*Aphis varians*) to each plant, with aphids sourced from a single colony from the valley in which the plant was to be deployed. At this time, plants were flowering and averaged 23 ± 1.6 cm in height. Aphids were placed among the flower buds, which is the location where they naturally feed. On 11 August we transported the potted plants and aphids to the ant nest mounds. We placed pairs of plants adjacent to each of the same focal nest mounds (n = 30 low and 30 high elevation plant pairs, for a total of 60) and randomly assigned one replicate per pair to either ant exclusion (with pots coated with fluon) or ant access treatments, with natural enemies allowed access to aphids in both treatments (Supplementary material Appendix 1 Table A1.1). Plants were watered every other day during the trial.

On 4–5 separate occasions from 11 to 25 August, we counted the aphids and ants on each plant. When data collection began, aphid colonies ranged in size from 6 to 67 aphids (27 ± 12 SD). We excluded from analysis the aphid colonies where ants were observed to have breached the exclusions, resulting in the following sample sizes (out of 30 colonies for each elevation and treatment): 13 and 15 colonies with ants excluded at low and high elevations (respectively), and 30 and 28 colonies open to ants at low and high elevations (respectively). By tracking aphid performance under ant exclusion at low versus high elevations, we evaluated the combined direct and indirect effects of elevation through altered top–down control by natural enemies (while controlling for plant quality). Comparing the performance of aphid colonies under ant exclusion versus ant access at low versus high elevations allowed us to evaluate whether elevation mediated the effects of tending ants on aphids.

To test whether elevation affected ant tending of aphids, we evaluated whether the presence and number of ants at aphid colonies differed with elevation. To assess ant presence

(versus absence) for aphid colonies in the ant access treatment, we used a GLMM with a binomial distribution. To evaluate ant number (for aphid colonies where ants were present; n = 29 and 18 at low and high elevations, respectively), we used a LMM. Ant number was calculated as the mean number of ants observed across all dates and was ln-transformed to improve the normality of residuals. Both models included the main effect of elevation, the number of aphids (averaged across all observations) as a covariate, and valley nested within elevation as a random effect (Supplementary material Appendix 1 Table A1.2). The model for ant number also included the elevation × aphid number interaction. A significant main effect of elevation would indicate an overall difference in ant tending while controlling for aphid abundance, and a significant elevation × aphid number interaction would indicate that elevation altered the ant per capita tending rate of aphids.

Furthermore, we evaluated the effects of elevation and ant tending treatment on aphid colony survival and growth. To assess survival (versus extinction), we used a GLMM with a binomial distribution, and to evaluate per capita growth rates (for surviving aphid colonies only; n = 23 and 6 with ants present and excluded [respectively] at low elevations and n = 19 and 10 with ants present and excluded [respectively] at high elevations), we used a LMM. Per capita growth rates were calculated as $r = [ln(N_t/N_0)]/t$, where N_0 is the initial population size at time $t = 0$ and N_t is the population size at the final observation, time $t = 13$–14 days. Both models included the main and interactive effects of elevation and ant tending treatment and the random effects of valley nested within elevation and ant mound nested within valley (Supplementary material Appendix 1 Table A1.2).

Natural enemy abundance

To additionally measure whether elevation mediated the top–down control of aphids by natural enemies, we evaluated the abundance of natural enemies at aphid colonies that were naturally occurring. In 2009 in one of the low (Spring Creek) and one of the high elevation valleys (East River Valley), we randomly selected two plants within each of 15 blocks spanning a distance of approximately 1 km. On six separate occasions from 4 to 15 August we counted the number of aphids, and on plants where aphids were present (n = 29 and n = 25 plants at low and high elevations, respectively), we counted the number of natural enemies on each plant (Supplementary material Appendix 1 Table A1.1). The natural enemies observed included hover fly larvae (Diptera: Syrphidae), ladybird beetles (Coleoptera: Coccinelidae), parasitoid wasps (Hymenoptera: Braconidae), predatory mites (Acari), and spiders (Araneae).

We evaluated whether the abundance of natural enemies differed between the low and high elevation valleys using a LMM. The mean number of natural enemies per plant across all observations (ln + 1 transformed to improve normality of residuals) was the response variable. The model included valley as a fixed effect, the mean number of aphids as a covariate, and block nested within valley as a random effect (Supplementary material Appendix 1 Table A1.2). While we use these analyses to provide information about whether natural enemy abundance potentially varies with elevation, our data are based on limited sampling, and it is possible that any observed differences would reflect valley-specific rather than elevational differences.

Data analysis procedures

We conducted all statistical analyses in R ver. 3.3.2 (<www.r-project.org>). To construct the LMMs, we used the 'lmer()' function in the 'lme4' package (Bates et al. 2015). For the GLMMs we used the 'glmmadmb()' function in the 'glmmADMB' package (Fournier et al. 2012). We calculated and compared AIC values using the 'AICtab()' function in the 'bbmle' package. Wald χ^2 tests with type III sums of squares were used to test for the significance of fixed effects in all models using the 'Anova()' function in the 'car' package (Fox and Weisberg 2010). In all models where interaction terms were not significant, we removed them to test for the significance of the main effects. We used the 'lsmeans()' function in the 'lsmeans' package to calculate least-squares means (LS-means) and conduct Tukey pairwise comparisons (Lenth 2016).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.kf2553j> (Nelson et al. 2018).

Results

Ant abundance and colony activity

Formica podzolica abundance in pitfall traps did not differ with elevation in 2012 or 2015 (LMMs: $\chi^2 = 0.006$, $p = 0.939$ and $\chi^2 = 0.254$, $p = 0.615$, respectively) (Supplementary material Appendix 3 Fig. A3.1). Moreover, although the focal ant mounds were larger (19%) on average at high elevations, this difference was not statistically significant (LMM: $\chi^2 = 0.642$, $p = 0.423$). Similarly, the number of ants on the surface of the ant mounds was greater at high elevations, though not significantly so (45%; LMM: $\chi^2 = 3.194$, $p = 0.074$).

Ant diet as indicated by stable isotope analysis

Elevation had no effect on ant diet assessed using stable isotopes, although some stable isotopes differed with ant life stage. We detected no effects of elevation on ant $\delta^{15}N$ (LMM: $\chi^2 = 1.197$, $p = 0.274$), C:N ratio (LMM: $\chi^2 = 2.435$, $p = 0.119$), $\delta^{13}C$ (LMM: $\chi^2 = 0.308$, $p = 0.579$), percent C (LMM: $\chi^2 = 0.295$, $p = 0.587$), or percent N (LMM: $\chi^2 = 0.709$, $p = 0.400$) (Fig. 2 and Supplementary material Appendix 3 Fig. A3.2). For adult ants (versus pupae),

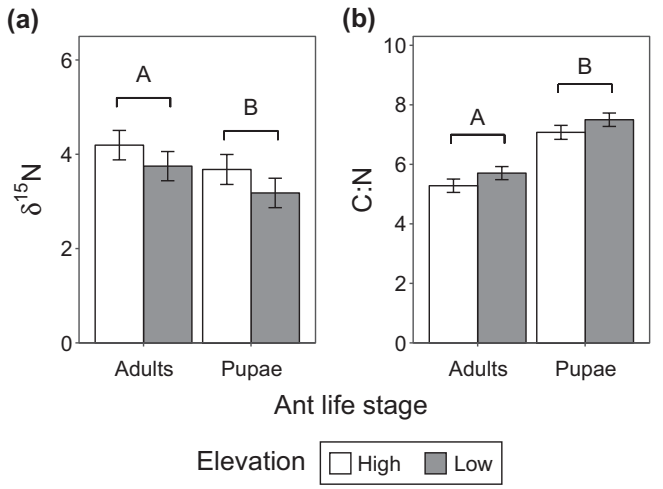

Figure 2. The mean (LS-means ± SE) (a) δ^{15}N and (b) C:N ratio of adult ants and pupae collected from ant mounds at both high and low elevations. Letters indicate significant differences among groups. For both the δ^{15}N and C:N ratio, there was no significant effect of elevation.

the δ^{15}N was 16% greater (LMM: $\chi^2 = 26.826$, $p < 0.001$) and the δ^{13}C was 1% greater (LMM: $\chi^2 = 4.637$, $p = 0.031$), whereas the adult (versus pupa) C:N ratio was 25% lower (LMM: $\chi^2 = 56.393$, $p < 0.001$) due to a 39% increase in percent N for adults (LMM: $\chi^2 = 55.723$, $p < 0.001$) but no change in percent C (LMM: $\chi^2 = 1.442$, $p = 0.230$) (Fig. 2, Supplementary material Appendix 3 Fig. A3.2).

Ant carbohydrate consumption as indicated by recruitment to baits

Ant colony bait consumption rates depended on the elevation × sugar concentration interaction (LMM: $\chi^2 = 10.748$, $p = 0.005$), where consumption rates increased more rapidly with sugar concentration at low elevation mounds (Fig. 3a). There were also significant main effects of elevation (2.3-fold greater at low elevations; LMM: $\chi^2 = 4.852$, $p = 0.028$), sugar concentration (LMM: $\chi^2 = 60.573$, $p < 0.001$) (Fig. 3a), and date (LMM: $\chi^2 = 68.024$, $p < 0.001$). However, forager abundance in the baits did not depend on an elevation × sugar concentration interaction (GLMM: $\chi^2 = 1.072$, $p = 0.585$), the main effect of elevation (GLMM: $\chi^2 = 2.072$, $p = 0.150$), or date (GLMM: $\chi^2 = 2.138$, $p = 0.343$), although forager abundance significantly increased with the sugar concentration (GLMM: $\chi^2 = 60.592$, $p < 0.001$) (Fig. 3b). Thus, similar to bait consumption rates, consumption rates per observed ant depended on the elevation × sugar concentration interaction (LMM: $\chi^2 = 9.315$, $p = 0.009$) as well as the main effects of elevation (1.9-fold increase; LMM: $\chi^2 = 4.818$, $p = 0.028$), sugar concentration (LMM: $\chi^2 = 37.010$, $p < 0.001$), and date (LMM: $\chi^2 = 63.656$, $p < 0.001$) (Fig. 3c). When we corrected for multiple comparisons using the Bonferroni method, all results were qualitatively identical.

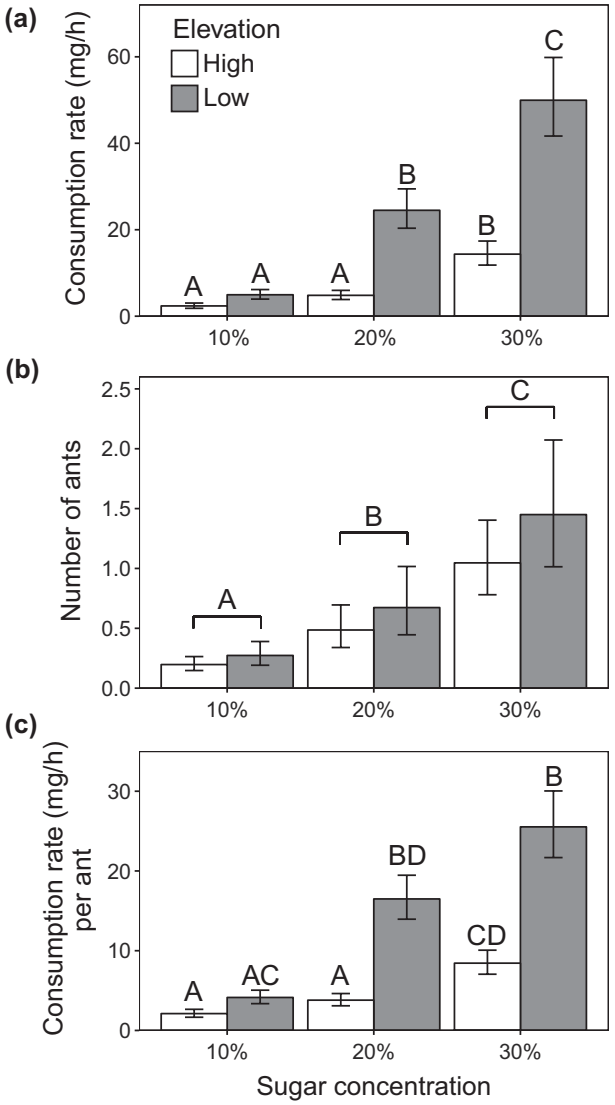

Figure 3. The mean (back-transformed LS-means ± SE) (a) bait consumption rates (mg/h), (b) number of ants observed feeding in the baits, and (c) consumption rates per observed ant (mg h^{-1} ant^{-1}) at high and low elevations (with replicates averaged across three dates). Letters indicate significant differences among groups.

Effect of ants on aphids

Similar to ant bait consumption, the proportion of aphid colonies tended by ants was 52% greater at low elevations (binomial GLMM: $\chi^2 = 7.030$, $p = 0.008$) but did not depend on the number of aphids in a colony (binomial GLMM: $\chi^2 = 2.114$, $p = 0.146$) (Fig. 4a). For aphid colonies that were ant tended, the number of tending ants increased with the number of aphids (LMM: $\chi^2 = 17.270$, $p < 0.001$) but did not depend on the elevation × aphid number interaction (LMM: $\chi^2 = 1.983$, $p = 0.159$) or the main effect of elevation (LMM: $\chi^2 = 1.567$, $p = 0.211$) (Fig. 4b).

Aphid colony survival depended on the elevation × ant treatment interaction (binomial GLMM: $\chi^2 = 7.370$,

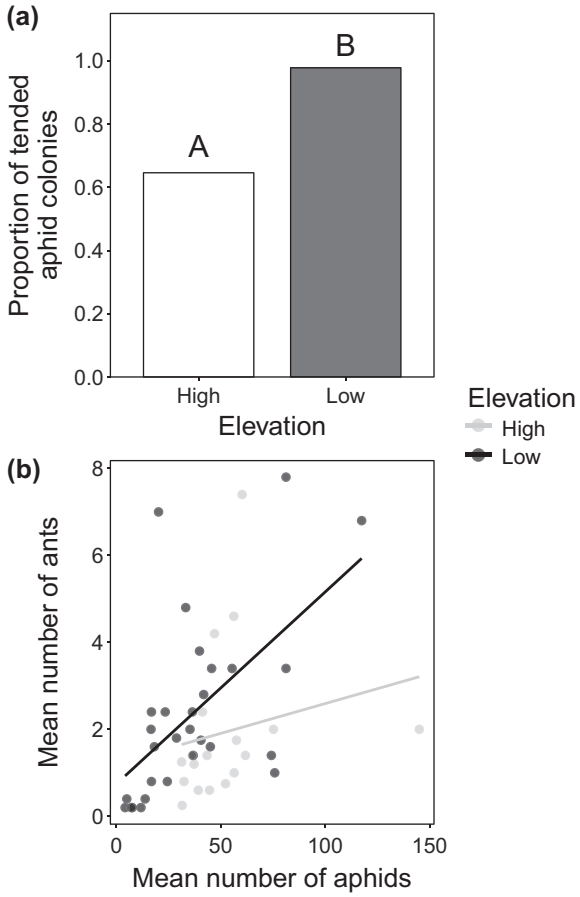

Figure 4. (a) The proportion of aphid colonies that were ant tended (the asterisk indicates a significant effect) and, where ants were present, (b) the relationship between the mean number of ants and the mean number of aphids per colony at high and low elevations. While separate trend lines are shown for low versus high elevations in panel (b), neither the aphid number × elevation interaction nor the main effect of elevation were statistically significant.

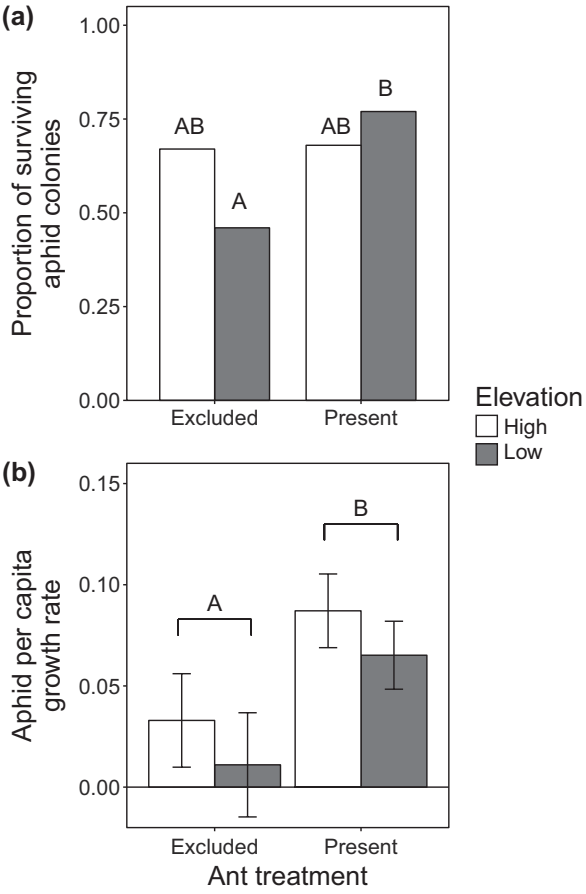

Figure 5. At high and low elevations where ants were excluded or allowed access ('present'), (a) the proportion of aphid colonies that survived during the experiment and (b) the mean (back-transformed LS-means ± SE) per capita growth rates of the aphid colonies that survived. Letters indicate significant differences among groups.

$p = 0.007$), where ants increased survival by 66% at low elevations (GLMM: $\chi^2 = 8.473$, $p = 0.004$) but had no detectable effect at high elevations (GLMM: $\chi^2 = 0.003$, $p = 0.960$) (Fig. 5a). There was also a significant main effect of elevation (binomial GLMM: $\chi^2 = 3.955$, $p = 0.047$), but the main effect of ant treatment was not statistically significant (binomial GLMM: $\chi^2 = 0.002$, $p = 0.963$) (Fig. 5a). For the aphid colonies that survived, ants doubled their per capita growth (LMM: $\chi^2 = 4.538$, $p = 0.033$) (Fig. 5b). However, per capita growth rates did not depend on an elevation × ant treatment interaction (LMM: $\chi^2 = 0.078$, $p = 0.780$) or the main effect of elevation (LMM: $\chi^2 = 0.922$, $p = 0.337$) (Fig. 5b).

Natural enemy abundance

Aphid natural enemies were 1.4-fold more abundant at the low elevation site (LMM: $\chi^2 = 9.627$, $p = 0.002$), but natural enemy abundance did not depend on the mean number of aphids per plant (LMM: $\chi^2 = 0.746$, $p = 0.388$) (Supplementary material Appendix 3 Fig. A3.3).

Contrasting analytical approach

Supplemental statistical analyses (presented in Supplementary material Appendix 2) were conducted based upon mean summer temperature (in place of analyses based upon elevation as high versus low). Although these analyses are pseudo-replicated, with the 10 replicates within a valley having only two to four estimates of mean summer temperature, they nonetheless provide some mechanistic insight into the factors driving elevational differences. With the exception of analyses of ant stable isotopes, the outcomes of these two approaches were qualitatively identical, with increasing temperature having the same effects as low (versus high) elevation (Supplementary material Appendix 2). In the stable isotope analysis, we found that the C:N ratio (but no other measure of stable isotopes) significantly increased with mean summer temperature (LMM: $\chi^2 = 3.936$, $p = 0.047$) (Supplementary material Appendix 2 Fig. A2.1).

Discussion

Low (versus high) elevations had stronger predator effects and even stronger ant tending effects, which together resulted in a stronger ant–aphid mutualism. These results are consistent with predictions for stronger species interactions at lower elevations and for the progressive sensitivity of trophic levels to temperature. Low elevation sites were associated with a higher natural enemy abundance, and in the absence of ants, lower aphid colony survival, supporting the prediction for stronger predator effects under warmer conditions. At the same time, low elevation sites were associated with ants consuming more carbohydrates and tending aphids more frequently, and in the presence of ants, higher aphid colony survival. These findings are thus consistent with the prediction for even stronger effects of ants (versus natural enemies) under warmer conditions (Voigt et al. 2003, Mooney et al. 2016). As a result of aphid colonies at low elevations having both lower survival without ants as well as higher survival with ants, ants increased aphid colony survival by 66% at low elevations but had no effect at high elevations. Furthermore, our supplementary analyses suggest that these effects were driven by increases in mean summer temperature. Accordingly, our findings support the notion that elevational gradients in multi-trophic interactions are driven by the progressive sensitivity of trophic levels to warming (Kishi et al. 2005, Barton et al. 2009).

Consistent with other studies of elevational gradients, we found a higher abundance of natural enemies at low elevations. Natural enemy abundance and diversity is often found to vary along environmental gradients (Hodkinson 2005) and frequently declines with increasing elevation and latitude (Straw et al. 2009, Sam et al. 2015, Moreira et al. 2018). Similar to our study, Straw et al. (2009) found that invertebrate predators of aphids were most abundant at low elevation sites, possibly causing aphids to be less abundant at low (versus mid) elevations. Importantly, such variation in predator effects may also cascade down to affect lower trophic levels. For example, Barton et al. (2009) found that warming increased the strength of the indirect effects of predators on terrestrial plant biomass. Likewise, Kishi et al. (2005) found that temperature altered predator foraging activity, resulting in cascading effects on lower trophic levels in an aquatic system.

Surprisingly, changes in abiotic conditions associated with elevation did not affect *Formica podzolica* ant abundance, as assessed using pitfall traps. In general, ants are known to be more abundant and diverse at lower elevations (Lessard et al. 2007, Sanders et al. 2007, Machac et al. 2011), and such patterns have been found in other sites near the RMBL (Menke et al. 2014). Moreover, previous studies have found ant mutualisms and the effects of ants as predators to be stronger at lower elevations and latitudes as the result of increases in ant abundance or changes in ant species composition (Koptur 1985, Olmstead and Wood 1990, Zelikova et al. 2008, Pellissier et al. 2012, Sam et al. 2015, Plowman et al. 2017), including along even relatively short elevational gradients such as that studied here (Binkenstein et al. 2017).

In contrast, elevation mediated ant demand for carbohydrate-rich resources, and as a result, ants tended aphids more frequently at low elevations. Stable isotope analysis of ants has previously demonstrated variation in ant diet among colonies of the same species within a single population (Mooney and Tillberg 2005, Tillberg et al. 2006) and along elevational gradients (Fiedler et al. 2007). While we did not detect an effect of elevation on ant stable isotopes, ant C:N ratios increased with mean summer temperatures (associated with low elevations), suggesting increased consumption of carbohydrates versus proteins. In addition, ants consumed more carbohydrate baits (per colony and per forager) and tended aphids more frequently at low elevations. Collectively, these results suggest that ants have a faster metabolism at low elevations where the climate is warmer, causing them to forage at a faster 'tempo' (sensu Davidson 1997). This conclusion is consistent with previous observations of variation in ant nutrient use along environmental gradients (Kaspari et al. 2008, Peters et al. 2014). However, further manipulative experiments are needed to determine the exact physiological and abiotic mechanisms underlying these changes in ant resource consumption. Possible factors include the direct effects of temperature, the availability of water or other carbohydrate resources, growing season length, and ant metabolic and nutritional demand (Cros et al. 1997, Cassill and Tschinkel 1999, Grover et al. 2007, Dussutour and Simpson 2009, Petry et al. 2012, Frizzi et al. 2016).

We did not investigate whether abiotic variation between high and low elevation sites mediates aphid performance directly or indirectly through changes in plant quality, but past studies suggest that such dynamics may not be important (Barton et al. 2009). For example, Mooney et al. (2016) found that variation in the abundance and performance of the aphid *Aphis helianthi* between sunny meadow and shaded understory environments was not due to variation in the direct effect of temperature or in host plant quality (*Ligusticum porteri*), but rather was mediated entirely by variation in the mutualist services provided by ants. Similarly, Nelson et al. (unpubl.) found that variation in the abundance and performance of the aphid *Pterocomma beulahense* between high and low elevation sites in the same region was not due to variation in the direct effect of temperature or host plant quality (*Populus tremuloides*), but was mediated by changes in the activity of mutualist ants. These past studies, in combination with the results provided here, thus suggest that the most significant consequences of variation in the abiotic environment for herbivore performance may be through effects on higher, and not lower trophic levels.

Because ants are dominant members of most terrestrial communities, elevational variation in ant activity can have widespread ecological consequences. In our study sites, *F. podzolica* engages in protection mutualisms with other honeydew-producing hemipterans (e.g. the aphids *A. helianthi* and *A. salicariae* on fireweed as well as hemipterans on other host plants) and extrafloral nectar-producing plants (e.g. *Helianthella quinquenervis*) (Addicott 1978, Inouye and Taylor 1979). Nelson et al. (unpubl.) found that because ants

at low elevations were more active and tended the aphid *Pt. beulahense* more frequently, aphid abundance was greater at low elevations. It is likely that differences in ant activity shape the distribution and abundance of many such mutualist species along this elevational gradient. Because ant protection mutualisms are considered to be 'keystone interactions' that have widespread effects on community structure (Styrsky and Eubanks 2007), variation in ant protection may also affect communities of associated species along environmental gradients. Moreover, because ants serve important roles as predators, nutrient recyclers (Griffiths et al. 2018), and seed dispersers (Giladi 2006), it is likely that variation in ant activity along abiotic gradients affects multiple ecosystem processes.

In summary, the results of this study are consistent with the prediction for a trophic gradient in sensitivity to abiotic change (Petchey et al. 1999, Voigt et al. 2003, Vasseur and McCann 2005) that in turn results in a gradient in interaction strength. These findings are consistent with the studies of two other ant-aphid systems in the same region (*Aphis helianthi* on the host plant *Ligusticum porteri*, Mooney et al. 2016, and *Pterocomma beulahense* on the host plant *Populus tremuloides,* Nelson et al. unpubl) that show evidence for temperature effects on higher trophic levels (predators and ants) but not on plant quality or aphid performance. Thus, this work highlights that in order to predict the consequences of climate change across entire food webs, it is important to understand the causes and consequences of trophic gradients in sensitivity to abiotic change.

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Conflicts of interest – The authors declare that there were no conflicts of interest.

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